

#1636

THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

RECEIVED
FEB 3 1975
ALBUQUERQUE

Date: 1/29/75

1. Principal Investigator (give title and degrees):

- 1) Ze'ev Trainin, Dr. Med. Vet. Head, Department of Immunology
- 2) Eitan Bogin, Ph.D., Head, Department of Biochemistry
- 3) Filip Neumann, Dr. Med. Vet. Ph.D., Department of Pathology

2. Institution & address:

Kimron Veterinary Institute, affiliated with Tel-Aviv University,
Bet Dagan, P. O. B. 12, Israel

3. Department(s) where research will be done or collaboration provided:

Department of Immunology, Kimron Veterinary Institute
" Biochemistry, " "
" Pathology, " "

4. Short title of study:

Round heart disease in turkeys as an animal model for α_1 -antitrypsin deficiency in man.

5. Proposed starting date: July 1, 1975

6. Estimated time to complete: Three years

7. Brief description of specific research aims:

In man, α_1 -antitrypsin (α_1 AT) deficiency is known to be hereditary and has been well documented. The accumulated data indicate that α_1 AT deficiency leads to a liver cirrhosis and chronic obstructive lung disease (COLD). The absence of a suitable animal model has limited the extent of research carried out on the disease.

Round Heart disease (RHD) of turkeys seems to be the suitable animal model. In the last two years (1973-4) workers at the Departments of Pathology and Immunology have found similarities between RHD of turkeys and α_1 AT deficiency in man.

Specific intracytoplasmic liver cell globules were found in RHD of turkeys. These globules are histologically and histochemically identical to those found in α_1 AT deficiency in man. These globules contain alpha globulins as was demonstrated by immunofluorescent technique. Blood analyses of turkeys with RHD showed a reduction of total serum proteins and a characteristic alpha globulin deficiency. Furthermore, serum of diseased turkeys showed a deficiency in the antitryptic activity.

All these data suggest the similarity between RHD of turkeys and α_1 AT deficiency in man.

This project seeks to prove the value of the turkey model by:

- a. Hereditary studies - identification of the different genotypes in relation to the proteinase inhibitor.

1003546358

- b. The physical and biochemical characterization of the proteinase inhibitor in turkeys' serum and its specificity towards different proteinases.
- c. Showing relationship between α_1 AT deficiency and the appearance of the syndrome.
- d. The effect of different environmental conditions by exposure of the birds to different factors (temperature, pollution etc.)

1003546359

Round Heart Disease in turkey poultts seems to be in individuals with low serum trypsin inhibition capacity (STIC) values. This may be caused either by defective inhibitor, by blocked release mechanism in the liver cells that produce the inhibitor or by a lower production of the inhibitor. The cause of the lower STIC is hereditary and the development of the RHD is conditioned by other factors such as temperature, air and water pollution, nutritional imbalance, administrated drugs or a combination of these all.

The etiology of RHD in turkeys shows a close similarity with the α_1 AT deficiency and the development of obstructive lung emphysema in man. A clarification of the hereditary mechanism of RHD in turkey poultts will possibly help in the understanding of α_1 AT deficiency in man. Knowledge of proteinase inhibitors and their function will enable a better understanding of the causes and development of the diseases, their followup and possibly their treatments.

9. Details of experimental design and procedures (append extra pages as necessary)

A. A survey will be made in turkey flocks to study the frequency of RHD, relative frequency of different STIC levels and proteinemia. The survey will be carried out by:

- Examination of all dead poultts
- Taking blood from a random sample from each flock, and testing for the following parameters:
 - Serum protein fractions by means of immunodiffusion tests
 - STIC levels
 - Total serum protein levels
 - Serum globulin levels

B. The electrophoretic pattern of turkey serum proteins will be studied in order to find the existing phenotypes. It is intended to investigate the possibility of establishing a reliable diagnostic test in order to identify poultts with RHD before their death. The methods which will be used are immunoelectrophoresis, immunodiffusion and immunofluorescence.

C. An experimental flock of turkeys will be established in the institute farm from birds which will be collected from infected flocks. The birds will be grouped according to STIC levels and crosses will be made between difference groups determined by their α_1 AT serum levels. Egg samples will be reserved from each cross and hatched. Serum types will be defined in the progeny of each cross to find the particular mode of inheritance of the different groups.

D. A followup of each group will consist of determining causes of death and pathological changes during their growth and development. Birds will be sacrificed and their liver, heart and lungs will be checked histologically and enzymatically. Blood will be taken from the birds and various serum enzyme levels will be determined in an attempt to monitor any internal changes.

1003546360

E. Poultts of different phenotypes will be exposed to different stresses such as temperature extremes, nutritional imbalances, crowding and pollution in an attempt to find genetic and environmental interactions and factors influencing the development of RHD.

F. The inhibitor α_1 AT from turkeys' serum will be isolated and characterized physically and chemically (MW, PH, sub-units, terminal amino-acids, amino-acids composition and conjugated moiety such as carbohydrates). The specificity of the isolated inhibitor will be checked against different proteases especially those found in the heart and lungs.

G. Inhibitor from diseased turkey poults will be isolated from both serum and liver and be compared with the inhibitor isolated from normal poults; this will enable us to learn whether the problem is in the synthesis of the inhibitor or its release to the blood.

Time schedule of research program:

- First year:
- a. Survey of sample flocks
 - b. Study of normal-STIC level in turkeys
 - c. Electrophoretic analysis of turkey serum
 - d. Isolation of proteinase inhibitor and characterization from turkey serum
 - e. Establishment of the experimental flock
- Second year:
- a. Analysis of crosses in the experimental flock
 - b. Histologic and enzymatic study of diseased poults
 - c. Isolation and characterization of the inhibitor in healthy and deficient turkeys
- Third year: Study of environmental factors that influence the outbreak of disease.

1003546361

3.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The research will be conducted at Israel's Kimron Veterinary Institute, Bet Dagan. The research will be done in collaboration by three departments - Immunology, Biochemistry and Pathology. All equipment and facilities will be available for this research.

Some of the equipment available for the research are:

- Ultracentrifuge
- High speed refrigerated centrifuges
- Spectrophotometers
- Fraction collectors
- Sonicators
- Oxygenograph
- Equipment for chromatography
- " " electrophoresis
- Scintillation and strip counters
- Gas chromatography
- Atomic absorption spectrophotometer
- Microscopes (including fluorescence)
- Standard equipment for histological preparation

In addition there are facilities in the institute farm for keeping and maintaining the experimental flock.

Additional studies will be carried out in collaboration with investigators at the City of Hope Medical Center, Duarte, California. (Please see

11. Additional facilities required: accompanying application for details)

Spectrophotometer - VIS U.V. digital readout

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

1003546362

12. Biographical sketch

Ze'ev Trainin

Title: Head, Immunology Dept.

REDACTED

Place of birth: Affula, Israel

Education: M.D.V. University of Bern, Switzerland - 1963

Dr. Med. Vet. " " " " 1966

Honors: A. Kimron Prize for research in bovine leukemia - 1969

A. Kimron Prize for research in Round Heart disease in turkeys - 1974

Major research interest: Immunological deficiencies in newborn and malignant processes

Research and/or professional experience:

Head, Dept. of Immunology, Kimron Veterinary Institute, Bet Dagan, Israel 1970-present

Head, Section of Immunology and Serology, Dept. of Bacteriology,

Kimron Veterinary Institute, Bet Dagan, Israel 1967-1969

Assistant Dept. of Microbiology, School of Veterinary Medicine, University of Bern.

1964-1966

Assistant Dept. of Pathology, School of Veterinary Medicine, University of Bern,

8/63 - 12/63

1003546363

13. Publications Ze'ev Trainin

Trainin, Z., Meirom, R. Calf immunoglobulins and congenital malformation.
Res. Vet. Sci. 15, 1 (1973).

Trainin, Z., Klopfer, U., Meidan, G. and Meirom, R. Levels of IgM and IgG in
the serum of normal and leukemic calves. J. comp. Path. 83, 115 (1973).

Meirom, R., Trainin, Z., Barnea, A., Neumann, F., Klopfer, U., Nobel, T.A.,
Disson, M.S. and Plener, O. Hypoproteinemia and alpha globulin deficiency
- in Round Heart disease of turkeys. Vet. Rec. 34, 262 (1974).

Neumann, F., Meirom, R., Nobel, T.A., Trainin, Z. and Klopfer, U. La maladie
du coeur rond des dindons conception etiologique actuelle.
Cah. med. Vet. 43, 100 (1974).

Meirom, R., Trainin, Z. and Neumann, F.

Alpha globulin intracytoplasmic hepatocyte globules in Round Heart disease
in turkeys, visualized by immunofluorescent technique. Poultry Sci. (In press)

1003546364

12. Biographical sketch

Eitan Bogin

Title: Head, Department of Biochemistry

REDACTED

Place of birth: Tel-Aviv, Israel

Education: Hebrew University, Jerusalem, Israel. B.Sc. 1956-1959)

University of California, Los Angeles, U.S.A. Biochemistry M.Sc. (1964)

" Ph.D. (1965)

Major research interest: Clinical biochemistry and its uses in veterinary medicine.
enzyme profiles of tissues and blood and changes due to diseases, nutritional status and effect of pollution.

Research and/or professional experience:

Head, Department of Biochemistry, Kimron Veterinary Institute, affiliated with
Tel-Aviv University Medical School. 1970 - present

Senior Lecturer, Tel-Aviv University Medical School 1972 - present

Assistant professor, University of Southern California Medical School (1969-1970)

Research Assistant - University of California, Los Angeles (U.C.L.A.) (1960-1965)

Lecturer, Negev University, Beer Sheva & graduate course in clinical biochemistry (1971)

Senior Lecturer, Tel-Aviv University Medical School. Faculty of Veterinary Medicine.

Two semesters course in:

Principles in general biochemistry and clinical biochemistry and its uses in
veterinary medicine - (1971- today)

Visiting Associate Professor, Institute for Anatomy, Physiology and Hygiene. Rhine
University, Bonn, Germany (1973)

Stipend 1961-1965. Isaac Loeb Foundation, Chicago, U.S.A.

Fellowship 1961-1965. University of California, Los Angeles, U.S.A.

Fellowship - Alexander von Humboldt

1003546365

13. Publications

Eitan Bogin

Bogin, E. and Ziv, G. Levels of LDH, GOT, ALP calcium, magnesium and phosphorus in normal and mastitic milk. Cornell Vet. 66, 666-676 (1973)

Bogin, E. and Hadani, A. Digestive enzymes in hard ticks. I. Proteolytic enzyme activity in the gut of Hyalomma excavatum female ticks. Z. Parasitenk. 41, 139-146 (1973).

Shlosberg, A., Bogin, E., Pipano, E. and Klinger, I. Serum enzyme levels in experimental theileriosis in calves. Refuah vet. 30, 24-27 (1973)

Bogin, E. The effect of deuterium oxide on oxidative phosphorylation of M. phlei. 1st Inter. Cong. of Bact.. 11p. 62 (1973)

Bogin, E. Studies on the effect of antibacterial compound from garlic on biological membranes. 9th Int. Cong. Biochem. p. 271 (1973).

1003546366

12. Biographical sketch. Filip Neumann

Title : Running Director, Dept. of Pathology

REDACTED

Place of Birth: Logoj, Rumania

Education: D.M.V. Faculty of Veterinary Medicine, Bucharest (Rumania) - 1948

Dr. med. Vet. Faculty of Veterinary Medicine, Bucharest - 1949

Ph.D. Hadassah Medical School, Hebrew University, Jerusalem - 1968

Honors: A. Kimron Prize for research in bovine leucosis - 1968

A. Kimron Prize, for research in Round Heart disease in turkeys -1974

Major research interest: Pathology of circulatory system.

Research and/or professional experience:

Research Veterinary Pathologist, Kimron Veterinary Institute, Grade A -
1971-present

W.H.O. Senior research training grant (Munich, Antwerpen, Cambridge)
1969-1970

Research Veterinary Pathologist, Kimron Veterinary Institute, grade B ,
1967-1971.

Veterinary Pathologist, Kimron Veterinary Institute, 1959-1967

Lecturer, Department of Veterinary Pathology, Faculty of Veterinary
Medicine, Arad (Rumania) 1954-1958.

Assistant, Dept. of Histology, Faculty of Veterinary Medicine, Arad,
Rumania 1949-1954.

1003546367

13. Publications

Filip Neumann

Neumann, F. The relationship between vasa sanguinea vasorum and aortic degeneration in the domestic swine. Path. Biol. 20, 437-441 (1972).

Neumann, F. and Ungar, H. Spontaneous aortic rupture in turkeys and the vascularisation of the aortic wall. Canad. vet. J. 14, 136-138, 1973.

Neumann, F., Nobel, T.A. and Klopfer, U. Calcinosis in goats. J. comp. Path. 83, 343-350 (1973).

Neumann, F., Klopfer, U., Nobel, T.A., Dison, M.S. and Bendheim, U. Peculiar liver changes in Round Heart disease in turkeys. Vet. Rec. 93, 599-601 (1973).

Neumann, F., Rattner, D., Klopfer, U. and Nobel, T.A. The vasa vasorum patterns in the fetal aorta of sheep. Angiologica, 10, 270-273 (1973).

Neumann, F., Meiron, Ruth, Nobel, T.A., Trainin, Z. and Klopfer, U. Round Heart disease in turkeys. A new etiological approach. Cah. med. vet. 47, 100-105, (1974)

1969-1970

1981-1982

1003546368

12. Biographical sketch Dan Rattner

REDACTED

Place of birth: Haifa, Israel. Path. Biol. 20, 437-441 (1972).

Education: B.Sc. Hebrew University, Jerusalem 1969

M.Sc. " " " 1971

Major research interest: genetics and biostatistics

S. 345-350 (1973).

Research and/or professional experience:

1970-71 Director of Animal Research farm Lahav affiliated with Kimron

Veterinary Institute

1972 - present Assistant in Dept. of Genetics, School of Agronomy, Hebrew

University, Jerusalem

13. Publications Dan Rattner

Rattner, D. A genetic comparison of quantitative traits in natural populations of the fungus *Schizophyllum commune*. M.Sc. thesis, Hebrew University, Jerusalem, 1971.

Neumann, F., Rattner, D., Klopfer, U. and Nobel, T.A. The vasa vasorum patterns in the fetal aorta of sheep. *Angiologica* 10, 270 (1973)

Rattner, D., Bedrak, E., Feinberg, J. and Francus, G. A note on Mastitis. Metritis an Agalactia syndrome in the sow.

1003546369

Francus, G. and Rattner, D. : On the relation between milk production and fertility in kibbutz dairy cattle herds. *J. Agric. Sci.* (In press).

14. First year budget:

A. Salaries (give names or state "to be recruited")

% time

Amount

Professional (give % time of investigator(s)
even if no salary requested)

Dr. Z. Trainin

20

Dr. E. Bogin

20

Dr. F. Neumann

20

D. Ratner

100

REDACTED

Technical help (animals)

50

3000

Sub-total

\$ 10500

Sub-Total for A

B. Consumable supplies (by major categories):

Animals (purchase & maintenance)

\$ 5000

Chemical and glassware

\$ 5000

Sub-Total for B

\$ 10,000

C. Other expenses (itemize).

Travel to U.S.

\$ 3000

Travel inland

\$ 1000

Sub-Total for C

\$ 4000

Running Total of A + B + C

\$24,500

D. Permanent equipment (itemize)

Spectrophotometer

\$ 3000

Sub-Total for D

\$ 3000

E

3675

Total request

\$31,175

E. Indirect costs (15% of A+B+C).

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	R	12,000	4,000	0	4,200	32,200
Year 3		13,500	4,000	0	4,650	35,650

1003546370

5.

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Checks payable to

Mailing address for checks

Principal investigator

Typed Name Dr. Z. Trainin

Signature [Signature] Date 26.12.74

Telephone
Area Code Number Extension

Responsible officer of institution

Typed Name Prof. A. Hadani

Title Director Kimron Veterinary Institute

Signature [Signature] Date 26.12.1974

Telephone Israel 03-940682
Area Code Number Extension

1003546371